Manufacturing and Characterization of 3-D Hydroxyapatite Bone Tissue Engineering Scaffolds

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ABSTRACT: Internal architecture has a direct impact on the mechanical and biological behaviors of porous hydroxyapatite (HA) implants. However, traditional processing methods provide very minimal control in this regard. This paper reviews a novel processing technique developed in our laboratory for fabricating scaffolds with controlled internal architectures. The preliminary mechanical property and *in vivo* evaluation of these scaffolds are also presented.

KEYWORDS: bioscaffolds; hydroxyapetite; bone tissue engineering

INTRODUCTION

Bone replacement has been an important subject in the the field of reparative medicine. One of the most-studied materials in bone replacement is porous hydroxyapatite (HA). The internal architectures, including pore size, pore shape, and pore connection pattern, play several important roles in the performance of the porous HA scaffolds. They control the degree of bone regeneration,[1] influence the path of bone regeneration,[2] and determine the mechanical properties of the scaffolds.[3] However, traditional processing methods, mainly hydrothermal exchange technique and organic particle embedding technique, provide very minimal control over the internal architecture of the scaffolds. To address this issue, we have developed a technique to implement designed internal architectures inside the HA scaffolds.[4] In this paper, we will briefly summarize the manufacturing and characterization of our 3-D HA bone tissue engineering scaffolds.

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MANUFACTURING

The manufacturing technique we have developed is a lost mold technique combining the use of (*a*) scaffold designs generated from computer-aided design (CAD) software or other imaging techniques, (*b*) thermal-curable HA/acrylate suspensions, and (*c*) epoxy molds made by stereolithography (SL). The application of SL in making epoxy models from computer-generated images have been depicted in detail in the literature[5] and will not be discussed here. To manufacture a 3-D HA scaffold the structure is first designed using CAD software or computer tomography (CT) data.[6] The negative image of the design is then used to build an epoxy mold on the stereolithography apparatus (SLA). A thermal-curable HA/acrylate suspension is subsequently cast into the epoxy mold and cured at 85°C. The cured part is placed in a furnace at high temperature to simultaneously burn out the mold and the acrylate binder. Left from the burn-out process is the HA green body in the designed 3-D structure. The HA green body is then fully sintered at 1350°C into a 3-D HA scaffold.

MECHANICAL PROPERTY EVALUATION

We first evaluated the mechanical property of our scaffolds. A cube with straight channels penetrating the cube orthogonally in X, Y, and Z directions was designed to mimic a simple three-dimensional interconnected structure. The cube was 7.4 mm in its width, length, and height. HA specimens were fabricated as described previously. The averaged channel size and total porosity of these specimens are listed in TABLE 1. The compressive strength of the specimens was investigated with an Instron machine (Instron 8521, Instron Corp. Canton, MA.). A total of twelve specimens were tested. The averaged compressive strength of the specimens was 30±8 MPa, comparable to that of the coralline HA of 25–35 MPa.[7] The averaged compressive modulus of the specimens from the stress–strain curve was 1.4±0.4 GPa.

IN VIVO EVALUATION

The *in vivo* performance of these 3-D HA scaffolds was also evaluated in miniature pigs. Two scaffold architecture designs were used in the *in vivo* study. The external geometry of both designs was identical: a cylinder with a diameter of 8 mm and a height of 6 mm. The orthogonal design contained channels of 450 μm penetrating in all X, Y, and Z directions. The radial design contained a central column of about 3 mm in diameter running in Z direction with channels of 380 μm extending from the center toward outside surface in radial directions. The channel size, wall size, and the final total porosity of these *in vivo* specimens are listed in TABLE 1.

Four Yucatan miniature pigs (6 months old) were used. Four defect sites were created in each hemi-mandible in the retromandibular area. Three defects on each side were filled with HA scaffolds, with the fourth site being left as either an empty control defect, or filled immediately with the cored bone to serve as a positive control. Two animals were sacrificed at 5 weeks and two at 9 weeks. The resected pieces were then embedded, sectioned, and stained with toludine blue. Quantita-

TABLE 1. The channel size, wall size, and total porosity of the mechanical and *in vivo* specimens

	Mechanical specimens	Orthogonal design	Radial design
Channel size (μm)	334 (width) 469 (height)	444	366
Wall size (μm)	500	748	
Porosity (%)	39	44	38

TABLE 2. Amount of bone regeneration divided by penetration zone and central zone

	Orthogonal design	Radial design
Penetration zone	55 ± 14%	41 ± 23%
Central zone	2 ± 2%	2 ± 3%

tive histomorphometry on the stained sections were performed using a square grid on a microscope.

In the retrieved specimens, normal regenerated bone tissue was found at 5 weeks and 9 weeks in both designs. Macroscopically, the geometry of the regenerated tissue was significantly influenced by the scaffold design. The regenerated bone tissue formed a single large piece at the center in the radial design, while the regenerated bone in the orthogonal design constituted an interpenetrating matrix with the HA scaffold. At 5 weeks, the average bone ingrowth was 19±9% in the orthogonal design and 14±3% in the radial design. At 9 weeks, the average bone ingrowth increased to 45±21% in the orthogonal design and 23±28% in the radial design. A further study[8] revealed that the distribution of the regenerated bone was not homogeneous among the tissue sections. A penetration zone of approximately 1.4 mm was found underneath the surface of both orthogonal and radial design scaffolds. The amount of bone regeneration corrected for the zone difference showed that the amount of bone regeneration was significantly different ($P < 0.05$) between the two zones (TABLE 2). Average bone regeneration in the orthogonal design was higher than the radial design, although not statistically significant, probably due to the relatively small number of animals (4) used in this pilot study. Larger-scale study is currently under way to investigate the effect of architectural design on the amount of bone regeneration.

CONCLUSIONS

HA scaffolds demonstrated biocompatibility and osteoconductivity in the *in vivo* animal model. The result indicates that the manufacturing process did not impose adverse effects on the biological property of HA.

The size and shape of the regenerated bone pieces were significantly different in orthogonal and radial designs. The result shows that it is possible to control the macroscopic morphology of the regenerated bone tissue through scaffold architecture design.

A 1.4-mm penetration zone was found underneath the surface in both orthogonal and radial design scaffolds at 9 weeks. Amount of bone regeneration between the penetration zone and the central zone was significantly different in both architecture designs. The result suggests that spatial distribution should be considered in evaluating the amount of bone regeneration inside the scaffolds.

No significant difference was found in the amount of bone regeneration between the two architectural designs, probably due to the small sample size.

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